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Prospective Study of Serological Conversion as a Risk Factor for Development of Leprosy among Household Contacts

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Although the prevalence of leprosy has declined over the years, there is no evidence that incidence rates are falling. A method of early detection of those people prone to develop the most infectious form of leprosy would contribute to breaking the chain of transmission. Prophylactic treatment of serologically identified high-risk contacts of incident patients should be an operationally feasible approach for routine control programs. In addition, classification of high-risk household contacts will allow control program resources to be more focused. In this prospective study, we examined the ability of serology used for the detection of antibodies to phenolic glycolipid I of *Mycobacterium leprae* to identify those household contacts of multibacillary leprosy patients who had the highest risk of developing leprosy. After the start of multidrug therapy for the index case, a new case of leprosy developed in one in seven of the 178 households studied. In households where new cases appeared, the seropositivity rates were significantly higher ($P < 0.001$) than those in households without new cases. Seropositive household contacts had a significantly higher risk of developing leprosy (relative hazard adjusted for age and sex [aRH], 7.2), notably multibacillary leprosy (aRH = 24), than seronegative contacts.

Over the past two decades, the conditions of leprosy control implementation have changed dramatically. This change is a result of the introduction of multidrug therapy (MDT) and the global effort to eradicate leprosy as a public health problem. The greatest impact has been through decreasing the registered prevalence of disease, thus freeing up control programs to concentrate on active cases (17).

At the beginning of the new millennium, leprosy control programs and the leprosy research community faced several new challenges. These related not only to changes in the prevalence of the disease, but also to changes in the contexts of leprosy control, such as those created by health sector reforms and other disease control programs. In conjunction with the absence of any evidence that incidence rates are declining (16), it is now clear that new approaches and strategies to definitely eradicate leprosy as a public health problem are required and should be linked to the epidemiological situation of the area (15).

It is well known that contacts of leprosy patients have an increased risk of developing leprosy compared to the general population (13). Several studies have shown that the majority of new patients have a contact relation with another patient (8, 14). This finding has led to development of a concentric circle model of transmission, similar to that of tuberculosis and that applied in the small pox eradication program (9). The model describes transmission radiating out from a patient in concentric circles among close contacts (14). It offers tools for improved leprosy control by refocusing control activities from the current blanket approach to a more focused and specific approach that includes intervention strategies applied to defined contacts. In their meta-analysis, Smith et al. (12) have shown that applying chemoprophylaxis to contacts is an effective way to reduce the incidence of leprosy and is more cost-effective when used for household contacts than for communities as a whole. Prophylactic treatment of contacts of incident patients may become an even more feasible approach under routine control program conditions when high-risk contacts can be identified and the expenditure of limited resources can be focused.

The presence of antibodies to phenolic glycolipid I (PGL-I) of *Mycobacterium leprae* in contacts has been repeatedly studied (11). However, to our knowledge serology has never been the focus of a long-term prospective study of multibacillary (MB) leprosy patients and their household contacts nor has it been viewed as a method for identifying incubating disease with an eye toward prevention. In this prospective study, we examined the ability of serology to identify those household contacts of multidrug-treated MB leprosy patients who had the highest risk of developing leprosy. Being able to make this distinction provides a basis for chemoprophylaxis and a new focus for control programs.

This study was conducted in an area where leprosy is endemic, in and around Cebu City, Cebu, The Philippines, from 1984 to 1996.

MATERIALS AND METHODS

Study population. Households of new MB leprosy patients were selected for entrance to the study based on accessibility and permanence in the Cebu area. The patients were selected from among those appearing at the Cebu skin clinic. MB leprosy patients were classified based on a bacterial index (BI) of 2 or greater

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TABLE 1. Accumulative distribution of new cases of leprosy among 178 households during periods of active and passive observation

Time interval	No. of new cases	No. (%)[a] of households with:		
		One case	Two cases	Any case
1985–1991 (Active surveillance)	27	21 (11.8)	3 (1.7)	24 (13.4)
1992–1996 (Passive surveillance)	6	0[b]	3 (1.7)	3 (1.7)
Total	33	21 (11.8)	6 (3.4)	27 (15.2)

[a] Percentage of 178 households.
[b] Three single-case households became two-case households and new single cases developed in three households, giving a net gain of zero for this category.

TABLE 2. Distribution of ELISA results among household contacts during active surveillance

ELISA result	No. of contacts	No. of new cases of leprosy		
		MB or PB	MB	PB
Positive[a]				
At entry	40	7	3	4
After conversion	59	7	6	1
Negative	460	13	1[b]	12
Total	559	27	10	17

[a] A positive ELISA result was defined as an OD at 492 nm of greater than 0.15.
[b] ELISA values obtained in 1985 were negative. After a lapse of 5 years between examinations (1986 to 1991), ELISA results were positive with an OD of 2.00 at diagnosis of MB leprosy (BI = 3.3) at the end of active surveillance in 1991.

as defined in 1984. Household contacts were examined and included in the study if they were found to be free of leprosy by clinical skin examination and had lived with the index patient for at least 24 months. A total of 193 MB leprosy patients representing 186 households with 601 contacts were enrolled from 1985 to 1988. Of the initial household contacts, 559 contacts residing in 178 households were included for analysis. The remaining 42 contacts (7%), who dropped out of the study, were similar in age, sex, and enzyme-linked immunosorbent assay (ELISA) result status to those who remained in the study (chi-square test; $P > 0.18$). Only contacts that could be monitored for 6 months or more were included in this study. The 559 household contacts consisted of 313 males with a median age of 21 years (interquartile range, 16 to 45 years) and 246 females with a median age of 27 years (interquartile range, 14 to 42 years). Duration in this study was measured in "person months" (number of months during which subjects in the study population have been exposed to the condition) to adjust for the various lengths of participation. Active surveillance was carried out continuously from 1985 to 1989 and again in 1991. Passive surveillance was continuously carried out at the Cebu skin clinic from 1985 to 1996.

ELISA. Sera were collected every 6 months for the first 4 years of the project, again in 1991, and sporadically between 1989 and 1996. The semisynthetic antigen natural disaccharide octyl bovine serum albumin, which mimics the PGL-I antigen of *M. leprae*, was used in the ELISA (3, 6). ELISA reactivity was considered to indicate positivity when optical density (OD) values exceeded 0.15. This cutoff value was based on data collected during the first year of the study from persons residing in the study area and determined by screening to be free of leprosy (6). The clinical staff was blinded to the ELISA results until a contact developed a case of disease, and the laboratory staff was blinded to the clinical results.

Statistical analyses. Statistical analysis focused on data collected from 1985 through 1991, which included the last point of active surveillance in 1991. Differences between household contacts with and without follow-up after study entry were investigated by using chi-square tests. The cumulative incidence of leprosy was calculated using the Kaplan-Meier product limit approach. Cox's proportional hazard analysis was performed using person months to estimate the risk of developing leprosy for household contacts with positive ELISA results and those with negative ELISA results. Two successive positive ELISA values were required for inclusion in this analysis. All statistical analyses were performed in SPSS 10.0.

RESULTS

Frequency of development of leprosy in households of MB leprosy patients. Household contacts of MB leprosy patients were prospectively monitored for the development of disease. The median OD value for the MB leprosy index cases in 178 households was 0.57 (interquartile range, 0.225 to 1.125). As can be seen in Table 1, contacts in approximately one (13.4%) of seven households of MB leprosy patients developed new cases of leprosy during the 7-year period of active surveillance. New cases developed in three households of MB leprosy patients during the period from 1992 through 1996, the interval of passive surveillance, resulting in an increase in the percentage of households in which leprosy was detected among contacts to 15.2%. Also during this time period, two cases occurred in 6 of 178, or 3.4%, of the households of MB leprosy patients.

The seropositivity rate was significantly higher among those contacts living in the households where new cases emerged ($n = 92$; 34.8%) than among the contacts living in households where no new cases were detected ($n = 467$; 14.3%; chi-square test; $P < 0.001$).

Table 2 provides a summary of the study population in relation to ELISA values and development of leprosy during active surveillance (1985 through 1991). As can be seen in the table, 40 of the 559 contacts were positive by ELISA at entry into the study and 59 became positive during active surveillance. Of the 27 contacts developing leprosy, 7 were positive by ELISA at entry, 7 became positive during active surveillance, and 13 remained negative by ELISA. All of the 10 new MB leprosy patients were or became positive by ELISA. Seven of these new patients were positive at the start of the study, and three converted from being negative by ELISA to being positive by ELISA. Five contacts developing paucibacillary (PB) leprosy were or became positive by ELISA, and contacts developing the remaining 12 PB leprosy cases never became positive by ELISA. All of the contacts who were positive by ELISA and eventually developed leprosy remained positive until development of disease. The maximum duration of seropositivity of contacts prior to diagnosis was 9 years.

Risk of developing leprosy. In order to adjust for variation in lengths of participation of subjects within the period of active surveillance, risk assessment for developing leprosy among contacts living in households of MB leprosy patients was performed using Cox's proportional hazard analysis as illustrated in Table 3. During the period of active surveillance, 27 (5%) of 559 contacts developed leprosy. The risks of development of MB or PB leprosy, MB leprosy only, and PB leprosy only were determined. Contacts who became positive by ELISA had a 7.65-fold-higher risk of developing MB or PB leprosy than contacts who were negative by ELISA. Contacts had a much higher risk of developing MB disease if they were positive by ELISA, with a relative hazard (RH) value of 34.4. The risk of developing PB disease was much lower, at an RH of 3.52. As can be seen in Table 3, all RH values were statistically significant. Adjustment for age and sex did not have a substantial effect on the RH values. The RH adjusted for age and sex

TABLE 3. Results of Cox's proportional hazard analysis of contacts developing leprosy and converting to ELISA-determined positive status prior to diagnosis

Disease	ELISA result	RH[a] (95% CI[b])	aRH (95% CI)
Any (MB or PB leprosy)	Positive	7.65 (3.53, 16.6)	7.15 (3.23, 15.8)
	Negative	1.00	1.00
MB leprosy	Positive	34.40 (7.14, 165.7)	24.00 (4.92, 116.7)
	Negative	1.00	1.00
PB leprosy	Positive	3.52 (1.24, 10.0)	3.80 (1.30, 11.1)
	Negative	1.00	1.00

[a] RH not adjusted for age and sex.
[b] 95% CI, 95% confidence interval.

(aRH) for MB or PB disease was 6.90, compared to the unadjusted value of 7.65. Multivariant analysis of RH related to classification by gender revealed that males had a higher, but not statistically significantly higher, risk of development of MB leprosy, with an RH of 4.51 (95% confidence interval, 0.93 to 21.8).

Seven contacts developing disease were positive by ELISA upon entry into the study, and 10 became positive during the study. Of the 68 contacts positive by ELISA who did not develop disease, none became negative during the course of the study. During the period of passive surveillance from 1991 to 1996, six additional cases of leprosy developed among these contacts of MB leprosy patients: one PB and five MB leprosy cases. The last two reported cases were of MB leprosy, and the contacts developing these cases had been in the study for 9 years and were also positive by ELISA for 5 and 9 years prior to detection of disease. The BIs for the passively detected MB leprosy cases were all between 4.0 and 5.0, with a median of 4.2. These BIs were in contrast to those for new MB leprosy cases discovered under active surveillance, which ranged between 1.3 and 4.7, with a median of 3.3, and were significantly lower (Mann-Whitney test; $P = 0.019$).

DISCUSSION

The current strategy for eliminating leprosy is based on the presumption that once the prevalence is below 1 in 10,000 on the global level, transmission will dwindle and eventually stop (6). However, newly diagnosed MB leprosy patients are thought to be a main source of infection, carrying a high bacterial load in their skin and being able to shed large numbers of bacteria from their nasal passages: 10^7 viable *M. leprae* bacteria per day on the average (5). It is thus very likely that these patients are contagious for a considerable length of time before their clinical diagnosis. Moreover, early lepromatous leprosy is often difficult to detect because clinical signs and symptoms are often delayed, which causes considerable delay in diagnosis. A method of early detection of those people prone to develop the most infectious form of leprosy may contribute to breaking the chain of transmission.

This study clearly establishes that anti-PGL-I antibody-positive household contacts of MB leprosy patients have a significantly higher risk of developing leprosy (aRH = 7.2), notably MB leprosy (aRH = 24), than seronegative contacts (Table 3). Seropositivity was also related to the development of PB disease (aRH = 3.8). Although serology is not a universal marker for PB disease, it does aid in discovery of patients with higher bacterial loads that are missed by skin slit smear examination (1). Interestingly, among the subset of PB patients within the leprosy spectrum, seropositive patients have a higher risk of treatment failure (1). In our study, we noted that five of the serologically positive new PB leprosy patients (Table 2) emerging from the contact population required retreatment and classification to MB leprosy status (results not shown), illustrating that seropositivity is associated with high bacterial loads in the patient. This is in accordance with results of previous studies showing that seropositivity is a better reflection of the total bacterial load than the BI for the skin (4, 7, 10).

It is well documented that a small percentage of the healthy noncontact population in areas where the disease is endemic may be serologically positive as well (3, 11). However, it is not clear that the antibody levels are persistent; our limited experience suggests that they are not. The study presented here shows that seroconversions in contacts are persistent among those who go on to develop disease.

Although there have been several cross-sectional studies which showed increased rates of seropositivity in contacts of leprosy patients compared to those in community controls (reviewed in reference 11), no prospective studies have been reported as far as we know. One retrospective serological study reported a lack of correspondence between seropositivity and development of leprosy (4). However, that study did not clearly define contacts in relation to the type of leprosy of the index case, neither the physical closeness of contact nor the duration of contact. Furthermore, the data presented in that study do not allow a calculation of RH of developing leprosy among seropositive contacts.

We observed that new patients diagnosed through passive case finding had significantly higher BIs than those actively diagnosed, which illustrates the transmission risks associated with delayed diagnosis. It is reasonable to conclude that MB leprosy patients are infectious long before their clinical diagnosis, since the majority of the new cases are diagnosed only years after the onset of disease and present with high BIs at diagnosis. We found that the maximum duration of seropositivity prior to diagnosis by passive ascertainment was 9 years, indicating the long incubation period prior to clinical diagnosis. This group of patients most likely pose a serious threat to the control of the transmission of leprosy, which is mainly based on case finding and ignores the long incubation period of MB leprosy cases.

New cases of leprosy developed in only one in seven households of MB leprosy patients after the treatment of the index patients was initiated (Table 1). Furthermore, in the house-

holds where disease did develop, there was a statistically significantly higher seropositivity rate than in the other households, thus demonstrating that some index patients and their families were more associated with transmission of infection than others and that serological testing of the contacts would allow for identification of the most important centers of infection in the community. In spite of the screening at entry into the study and the immediate application of MDT for the index cases, 33 new cases emerged among the contact population of 559 (27 cases during active surveillance and 6 cases during passive surveillance) in the 10-year follow-up period. This indicates that MDT, while effective for the index case, plays little role in prevention of new cases in the household once infection has been established.

Since there is no marker for infection, leprosy control programs currently have no tools other than clinical screening of household contacts. However, it is notable that early MB disease does not present with marked clinical signs. *M. leprae*-specific antibodies to PGL-I as a marker for bacterial load in patients have been well documented; antibody levels are associated with the spectrum of disease, decline upon treatment, and rise prior to relapse (reviewed in reference 11). Our results indicate that seropositive household contacts have a long-term risk of development of leprosy, comparable at least to the risk of developing tuberculosis among individuals with positive purified protein derivative skin test results. In general, most PB leprosy patients do not develop PGL-I antibodies and are not associated with the spread of the disease (13). Those PB leprosy patients with elevated antibodies should probably be treated as MB leprosy patients (1).

There are now several studies which clearly show that close contact is more important in transmission than often believed (8, 14). The risk of developing leprosy is greatest among close contacts of leprosy patients, like household contacts, but is also significant among neighbors and social contacts and in particular among close contacts of MB leprosy patients. Screening contacts of leprosy patients in order to find and follow-up with antibody-negative contacts and to treat antibody-positive high-risk household contacts with an MB leprosy treatment regimen should ultimately prevent transmission and opens the way for a rational program for eradication. This study shows that serology is a useful tool for this purpose. Recently, a simple lateral flow test for the detection of anti-PGL-I antibodies has been described (2), which can replace ELISA and extends serology to local leprosy control programs. This test provides a simple method for annual rescreening of serologically negative household contacts.

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